BTK Leu528Trp - a Potential Secondary Resistance Mechanism Specific for Patients with Chronic Lymphocytic Leukemia Treated with the Next Generation BTK Inhibitor Zanubrutinib


*contributed equally to the research
BTK inhibitors in CLL

- Highly efficacious class of agent in frontline and relapsed/refractory CLL (Byrd et al, NEJM 2014; Shanafelt et al, NEJM 2019; Woyach et al, NEJM 2018)

- BTKi covalently bind to Cys481 residue in BTK resulting in blocking of enzymatic activity

- “First generation” BTKi
  - Ibrutinib

- “Second generation” BTKi
  - Acalabrutinib, tirabrutinib, zanubrutinib

- Non-C481 dependent BTKi
  - Vecabrutinib, LOXO-305, ARQ-531

(Bond & Woyach, 2019)
Zanubrutinib in CLL

• Second generation BTK inhibitor

• Efficacious in treatment-naïve and relapsed/refractory CLL/SLL (Tam et al, Blood 2019)

• Multiple clinical trials in a variety of B-cell lymphoma subtypes ongoing

• Greater selectivity for BTK (over EGFR, ITK, and TEC) than ibrutinib (Tam et al, Blood 2019)
Resistance to BTK inhibitors in CLL

- BTK inhibitor (ibrutinib) resistance mechanisms
  (i) Drug-binding site Cys481 mutations (Cys481Ser, Cys481Phe/Arg/Tyr) (Woyach et al, NEJM 2014)
  (ii) Downstream activating PLCG2 mutations (Liu et al, Blood 2015)
Aim

- To investigate possible resistance mechanisms to the second generation BTK inhibitor zanubrutinib (ZANU) in patients with CLL
Cohort

- 38 patients with relapsed/refractory CLL treated with ZANU on clinical trials (NCT02343120, NCT02569476, NCT03336333, NCT02795182) at three centres in Melbourne, Australia

- Four of 38 patients had CLL progression on ZANU (time to progression 5, 26, 29 and 48 months)

- Amplicon next generation sequencing (NGS)
  - Targeted amplicon sequencing (sensitivity approx 3-5% VAF)
    - ARAF, BCL2, BIRC3, BRAF, BTK (exon 11, 15, 16), CARD11, CD79B, CXCR4, DNMT3A, EZH2, FOXO1, FYN, ID3, IDH1, IDH2, JAK3, KRAS, MAP2K1, MYD88, NOTCH1, NRAS, PHF6, PLCG1, PLCG2 (exon 16, 19-20, 24, 27-28), RHOA, RUNX1, SF3B1, STAT3, STAT5B, STAT6, TCF3, TP53, XPO1
<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Pre-ZANU</th>
<th>Post-ZANU</th>
</tr>
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<tbody>
<tr>
<td><strong>CLLZ1</strong></td>
<td>TP53 c.659A&gt;G; p.(Tyr220Cys)</td>
<td>TP53 c.659A&gt;G; p.(Tyr220Cys) BTK c.1441T&gt;A; p.Cys481Ser BTK c.1583T&gt;G; p.Leu528Trp</td>
</tr>
<tr>
<td><strong>CLLZ2</strong></td>
<td>BRAF c.1799T&gt;A; p.(Val600Glu) NOTCH1 c.7541_7542del; p.(Pro2514Argfs*4)</td>
<td>BRAF c.1799T&gt;A; p.(Val600Glu) NOTCH1 c.7541_7542del; p.(Pro2514Argfs*4) BTK c.1441T&gt;A; p.(Cys481Ser) BTK c.1442G&gt;C; p.(Cys481Ser) BTK c.1583T&gt;G; p.(Leu528Trp)</td>
</tr>
<tr>
<td><strong>CLLZ3</strong></td>
<td>No mutations detected</td>
<td>TP53 c.1125_1140del; p.(Ser376Lysfs*41) BTK c.1441T&gt;A; p.(Cys481Ser) BTK c.1442G&gt;C; p.(Cys481Ser) BTK c.1442G&gt;A; p.(Cys481Tyr) BTK c.1583T&gt;G; p.(Leu528Trp)</td>
</tr>
<tr>
<td><strong>CLLZ4</strong></td>
<td>BRAF c.1406G&gt;C; p.(Gly469Ala) XPO1 c.1711G&gt;A; p.(Glu571Lys)</td>
<td>BRAF c.1406G&gt;C; p.(Gly469Ala) XPO1 c.1711G&gt;A; p.(Glu571Lys) BTK c.1442G&gt;C; p.(Cys481Ser) BTK c.1583T&gt;G; p.(Leu528Trp)</td>
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</table>
Leu528Trp is detectable in zanubrutinib treated patients before clinical CLL progression.
Leu528Trp identified in 3 out of 34 patients on zanubrutinib in steady state

- ddPCR performed on 34 patients without disease progression but persistent measurable disease on zanubrutinib

- BTK Leu528Trp detected in 3 out of 34 patients (VAF <1%)
BTK Leu528Trp and Cys481 mutations are present in different cells in zanubrutinib progressors
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BTK Leu528Trp is rarely observed in patients treated with ibrutinib

<table>
<thead>
<tr>
<th>Author</th>
<th>BTK Leu528 codon assessed</th>
<th>Patients with progressive CLL</th>
<th>BTK Cys481 (and non-Leu528Trp)</th>
<th>BTK Leu528Trp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woyach et al, NEJM 2014</td>
<td>Yes</td>
<td>6</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Maddocks et al, JAMA 2015</td>
<td>Yes</td>
<td>19 (8 RT)</td>
<td>13</td>
<td>1</td>
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<td>Sharma et al, Oncotarget 2016</td>
<td>Yes</td>
<td>1</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Woyach et al, J Clin Oncol 2017</td>
<td>Yes</td>
<td>46</td>
<td>37</td>
<td>0</td>
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<tr>
<td>Gango et al, Int J Cancer 2019</td>
<td>Yes</td>
<td>20</td>
<td>8</td>
<td>0</td>
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<tr>
<td>Kanagal-Shamanna et al, Cancer 2019</td>
<td>Yes</td>
<td>29</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>121</strong></td>
<td><strong>83 (68.5%)</strong></td>
<td><strong>1 (0.8%)</strong></td>
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49 patients with progressive CLL on ibrutinib (European Research Initiative CLL [ERIC])
• Targeted next generation sequencing (Haloplex)
• 0/49 patients found to harbor the BTK Leu528Trp
BTK Leu528Trp mutation disrupts the binding pose of ibrutinib, zanubrutinib and tirabrutinib

Net change in binding free energy: Trp528 vs Leu528: 9.37 ± 0.38 kcal/mol

Net change in binding free energy: Trp528 vs Leu528: 10.04 ± 0.53 kcal/mol

Net change in binding free energy: Trp528 vs Leu528: 7.57 ± 0.34 kcal/mol
BTK Leu528Trp leads to abrogated kinase function in biochemical and cellular models
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Steric clashes

ATP

S/B

BTK(nM)

BTK C481S
BTK WT
BTK L528W/C481S
BTK L528W

HEK293 Con
BTK WT
BTK C481S
BTK L528W
BTK C481S/L528W

pBTK (Y223)
GAPDH
BTK Leu528Trp and downstream signalling

ATP

BTK → BTK → PLCG2 → PLCG2 → Proliferation/Survival
BTK Leu528Trp and downstream signalling

ATP

BTK

L528W

PLCG2

P

BTK

L528W

PLCG2

P

Proliferation/Survival
BTK Leu528Trp and downstream signalling

Heidorn et al, Cell 2010
Patient CLL cells harboring Leu528Trp show downstream activation of PLCG2
Patient CLL cells harboring Leu528Trp show downstream activation of PLCG2

**BTK WILDLTYPE**

**BTK Leu528Trp**
Patient CLL cells harboring Leu528Trp show downstream activation of PLCG2
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BTK WILDTYPE

BTK Leu528Trp

p-PLCγ2 (Tyr759)
Summary

• BTK Leu528Trp mutations are enriched in CLL progression on zanubrutinib compared to ibrutinib

• BTK Leu528Trp results in a marked impairment of binding of zanubrutinib (as well as ibrutinib and tirabrutinib) to BTK

• BTK Leu528Trp occurs with Cys481 mutations but is present in different CLL cells in the tumor compartment

• BTK Leu528Trp is associated with loss of native kinase function however downstream signalling pathways appear intact in patient CLL cells suggesting an alternative mechanism of PLCG2 phosphorylation
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